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Morphology and Behavior of an Unusually Flexible Thoracic Limb in the Snapping Shrimp, *Alpheus heterochelis*

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Abstract. The second thoracic limb in the snapping shrimp, *Alpheus heterochelis*, is much thinner, more elongated and flexible, and has a larger ganglion than its serial homologs. The greater length and flexibility is largely due to one of the limb segments—viz., the carpus—which consists of five separate segments, rather than the single segment typical of the other limbs. Externally, the multisegmented carpus is relatively free of cuticular projections except for scattered simple setae. The adjoining segments—the merus and the propus—are also smooth except for clusters of long simple setae on the pollex and dactyl. Internally, each of the carpal segments has three muscles—a bender, stretcher and rotator—all restricted to the distal half of the segment. In keeping with the sensillum-free exterior of the multisegmented carpus, only about 1000 axon profiles originate in the carpus out of a total of 6000 counted at the base of the ganglion. This total number is roughly half that found in the first thoracic limbs. Conversely, the number of axon profiles in the longitudinal connectives to the second thoracic ganglion is about 25% greater than that to the first thoracic ganglion and may partly account for the size difference between these two ganglia.

In terms of their behavior, the second thoracic limbs are almost constantly active, mostly probing the substrate, and occasionally grooming various body parts. Part of the probing behavior consists of food foraging and retrieval, especially from concealed and hard-to-reach locations. Because of their flexibility, these limbs are particularly adept at such movements.

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Introduction

Shrimps of the family Alpheidae are commonly referred to as snapping shrimp, or pistol shrimp, because of the audible popping sound they make when closing their major cheliped. The major or snapper cheliped is highly specialized both morphologically and physiologically, to produce this defensive response (Prizbram, 1901; Ritzmann, 1974). The opposite cheliped or claw, referred to as the minor or pincer claw, is smaller, less elaborate, and used primarily in burrowing behaviors. Although these paired claws have attracted much attention, because of their asymmetry and their capacity to reverse this asymmetry (Prizbram, 1901; Wilson, 1903), the next pair of thoracic limbs, which are also highly specialized in form and function, have been somewhat neglected.

The second thoracic limbs are bilaterally symmetrical and very thin compared to the chelipeds. The most striking morphological specialization is, however, the multisegmented carpus, which is made up of five separate segments rather than the single segment characteristic of all the other thoracic limbs in the shrimp and of crustacean limbs in general. The multisegmented carpus gives the second thoracic limb a high degree of flexibility. Indeed, in another snapping shrimp, *Alpheus pachychirus*, these second thoracic limbs are used as needles with which to stitch algal mats into a temporary retreat (Schmitt, 1975). Although this type of behavior is not seen in *Alpheus heterochelis*, the second thoracic limbs are, nevertheless, strikingly flexible in their movements. Moreover, these movements are not associated with walking, which is performed by the remaining three pairs of thoracic limbs, but with exploring the environment, feeding, and grooming. Thus

the second thoracic limbs make almost constant searching movements, by which their distal ends delicately explore the surrounding substrate.

Another interesting point about these highly flexible second thoracic limbs is that their ganglion appears to be larger than that of any of the other thoracic ganglia. This would imply that the volume of neural tissue concerned with the behavior of the second thoracic limb is greater than that of any other thoracic limb. We have therefore investigated the morphology and behavior of these limbs.

Materials and Methods

Adult snapping shrimps, *Alpheus heterochelis* (Say), were collected from tidal pools around Beaufort, North Carolina, and shipped to our laboratory in Scarborough, Ontario. In the laboratory, the animals were held in 25 l glass aquaria equipped with a bottom gravel filter and partitioned into 12 compartments with fiberglass screens. The aquaria were filled with artificial seawater that was kept at room temperature (22°C). A specially prepared diet—a blended mash of chicken livers and hearts and commercial dog food—was fed to the animals daily. The shrimps were sexed on arrival in the laboratory, and their molt history during captivity was recorded. Behavioral and morphological observations were made on adult animals obtained from the wild, and a few juveniles reared in our laboratory were used to supplement the morphology.

Morphology

Scanning electron microscopy. The second thoracic limbs of adult shrimps were removed by a gentle pinch, which induces the animal to autotomize the limb at its base. These isolated limbs (or in the case of juvenile shrimps, whole animals) were fixed for 1–3 h in a 0.15 *M* sodium cacodylate buffer (pH 7.4) containing 2.5% glutaraldehyde, 0.2% formaldehyde, 2 m*M* $CaCl_2$, 0.06 *M* NaCl, and 0.3 *M* sucrose. Next, the tissue was washed in 0.15 *M* cacodylate buffer, containing 2 m*M* $CaCl_2$, 0.06 *M* NaCl, and 0.3 *M* sucrose for 1 h, with several changes. Following dehydration in a graded ethanol series, the tissue was transferred into acetone, before being critical point dried, and mounted with silver paste on Cambridge SEM stubs. The tissue was sputter-coated with gold-palladium and examined with a Hitachi S-530 scanning electron microscope.

Transmission electron microscopy. The focus of this study was the muscles and nerves within the 2nd thoracic limb. Isolated limbs were pinned out in a dish and superfused with the fixative described previously. When the nerves at the base of the ganglion were to be studied, the thoracic nervous system was exposed on its ventral side *in situ* and superfused with fixative. Following an initial fixation of 1 h, the tissues were further dissected, and selected pieces were removed and allowed to fix for an additional hour. A rinse in buffer solution for 0.5 h followed, and the pieces of tissue were then post fixed in 2% O_3O_4 for 1 h. Next, the tissue was briefly rinsed in buffer solution, dehydrated in a graded ethanol series, cleared in propylene oxide, and embedded in Epon-Araldite (Pearce *et al.*, 1986).

Thin (75–100 nm) cross-sections of the nerve were taken close to the ganglion to capture all the axons between the limb and its hemiganglion. These sections were mounted on Formvar-coated single-slot grids, stained with uranyl acetate and lead citrate, and examined with a Zeiss 9S and a Siemens 102 electron microscope.

Cross-sections of the ganglionic nerves were photographed in their entirety via a series of overlapping exposures at 1800×. The resulting negatives were printed to 6000× and assembled into a montage in which the smallest, usually unmyelinated, axons (0.2 μm diameter) were distinct, and could be easily counted. A similar procedure was followed for the ventral nerve cord connectives, except that the initial exposures were at 500× and the final prints were at 3500×. At this magnification, individual axon profiles were easily recognized, as the overwhelming majority were myelinated and therefore relatively large.

Behavior

Observations were carried out on animals acclimated to conditions in the laboratory (room temperature, 10:14 L:D photoperiod) for at least one month. Two small observation tanks (32 cm × 9 cm × 16 cm) were set up as follows: the sides and back were covered with opaque paper, and the inside was partially filled with sloped gravel or sand, and half-filled with artificial seawater. The animals to be observed were placed in the observation tanks and allowed to acclimate overnight. Observations were carried out in the morning and afternoon, under artificial lighting.

Time budget. The focus of our reconnaissance observation was the flexible limb, and our ethogram was constructed with this in mind. We then determined the percentage of time occupied by the cataloged behavioral states using instantaneous sampling (Lehner, 1979). Behavioral states were scored at 20-s interval points throughout an 11-min observation period.

Foraging experiments. An experiment was designed to test the importance of the flexible limb in foraging, specifically for concealed food; *i.e.*, the foraging behavior of shrimp with intact flexible limbs (control) was compared to that of shrimp with missing flexible limbs. Males ranging in length (rostrum to telson) from 28 to 34 mm were used. The flexible limbs were autotomized and the shrimp

allowed at least a week to recover. The procedure appeared to cause little stress, as expected, because autotomy is a defensive adaptation and, indeed, shrimp are frequently found with missing limbs. Their general behavior appeared normal.

The trials for observing the foraging behavior were set up as follows: the individual to be tested was confined to one end of the tank with a piece of screen and, at the other end, a small piece of coral was partially buried. Into one of the corralites (approximately 5 mm deep × 2 mm wide) was placed a previously frozen brine shrimp, along with a generous squirt of "brine-shrimp water." The shrimp was released from confinement by removal of the dividing screen, and the time taken to locate the food and actually retrieve it were both recorded. In order for the trial to be valid, the animal had to demonstrate foraging behavior; *i.e.*, frantic crawling around with intense substrate probing. If the food was not located or found within 10 min, the trial was terminated. Each animal was tested twice daily, in the morning and afternoon.

Results

External morphology

Alpheus heterochelis bears five pairs of thoracic limbs: the first two pairs are chelated, and the remaining three pairs are not (Fig. 1A). The first pair of limbs is elaborated into chelipeds; they are bilaterally asymmetrical, one bearing a major (snapper) chela, and the other a minor (pincer) chela, both held in front of the animal. The remaining four pairs of thoracic limbs are not as elaborate, are much smaller, and are held to the side of the animal. The second pair of thoracic limbs differ from the others in that they are much thinner and have a multisegmented carpus. There are five carpal segments; the proximal two are much longer than the distal three, but the joint between each of the segments is in the same orientation as that between the most proximal segment and the merus (Fig. 1B). Consequently, at each joint the distal segment can lie in line with its proximal partner, or it can be bent to almost touch its proximal partner through an angle of 130–140°. This degree of bending at each of the four intercarpal joints makes this limb extremely flexible, allowing its chelated propus-dactyl segment to reach parts of the body otherwise inaccessible to a limb with a single segmented carpus.

The multisegmented nature of the carpus also makes it the longest segment of this limb, much longer than its counterpart in the other three posterior limbs. In contrast, the propus is much shorter in the second limb than in the remaining limbs.

Each of the carpal segments, as well as the more proximal merus, is smooth and bare (Fig. 1B) except for a few scattered short simple setae (Fig. 1C). The apical end of these simple setae are specialized into a sheath (Fig. 1D). The propus is also relatively free of cuticular projections (Fig. 1B) except for its most distal parts, the pollex and the dactyl (Fig. 1E). Situated at the distal end of the pollex and dactyl, on each of the medial and lateral aspects, are prominent clusters of long setae. Scattered more proximally are one or two smaller clusters. These long setae are serrulate in form and have an apical pore (Fig. 1F). Others have their tip elaborated into a sheath (Fig. 1G). A row of short setae occur along the closing edge of the dactyl (Fig. 1E) and these are serrulate and have an apical pore (Fig. 1H).

An opportunity to examine juvenile shrimps arose in our laboratory when a berried female hatched its eggs and we were able to rear these developing shrimps into juvenile forms. In the early juvenile stages, when the paired chelipeds have not yet differentiated into snapper and pincer types, the carpus in the second thoracic limb was fully differentiated with five segments on the one side but only four on the other (Fig. 2A). Of these four segments, the distal three were matched in shape, size, and location to their counterparts on the opposite limb, while the fourth, most proximal segment had not yet subdivided into two. Indeed, a short, shallow furrow on its ventral side (Fig. 2B, C) seemed to mark the formation of a new segment. This observation suggests that the segmentation of the carpus may occur in a distal to proximal direction; possibly the carpus arises as a single unit initially and subsequently becomes segmented.

No sensilla occur on the carpal segments at this juvenile stage; only a single cluster of long setae was present at the distal tip of the pollex and dactyl (Fig. 2A).

Internal morphology

Muscle elements. The musculature in the propus and carpus was examined in thick and thin cross-sections of these segments. The propus typically has two muscles, a small opener and a large closer muscle. Both originate on the exoskeleton and insert via apodemes to the dactyl, which opens and closes in response to contraction of the respective muscles. The fine structure of these muscles is typical of other crustacean striated muscles (Govind and Atwood, 1982) and of the snapping shrimp claw closer muscle (Mellon and Stephens, 1980); *viz.*, myofibrils composed of serially repeating sarcomeres, in which thick filaments are surrounded by thin filaments. We did not further characterize these muscles into fiber types. Mitochondria were found typically around the periphery of the fiber, where they formed a relatively thick rind. They also occurred occasionally interspersed within the fiber, usually in a single row separating myofibrils.

Both muscles also occasionally displayed nerve terminals that resembled those previously described in the

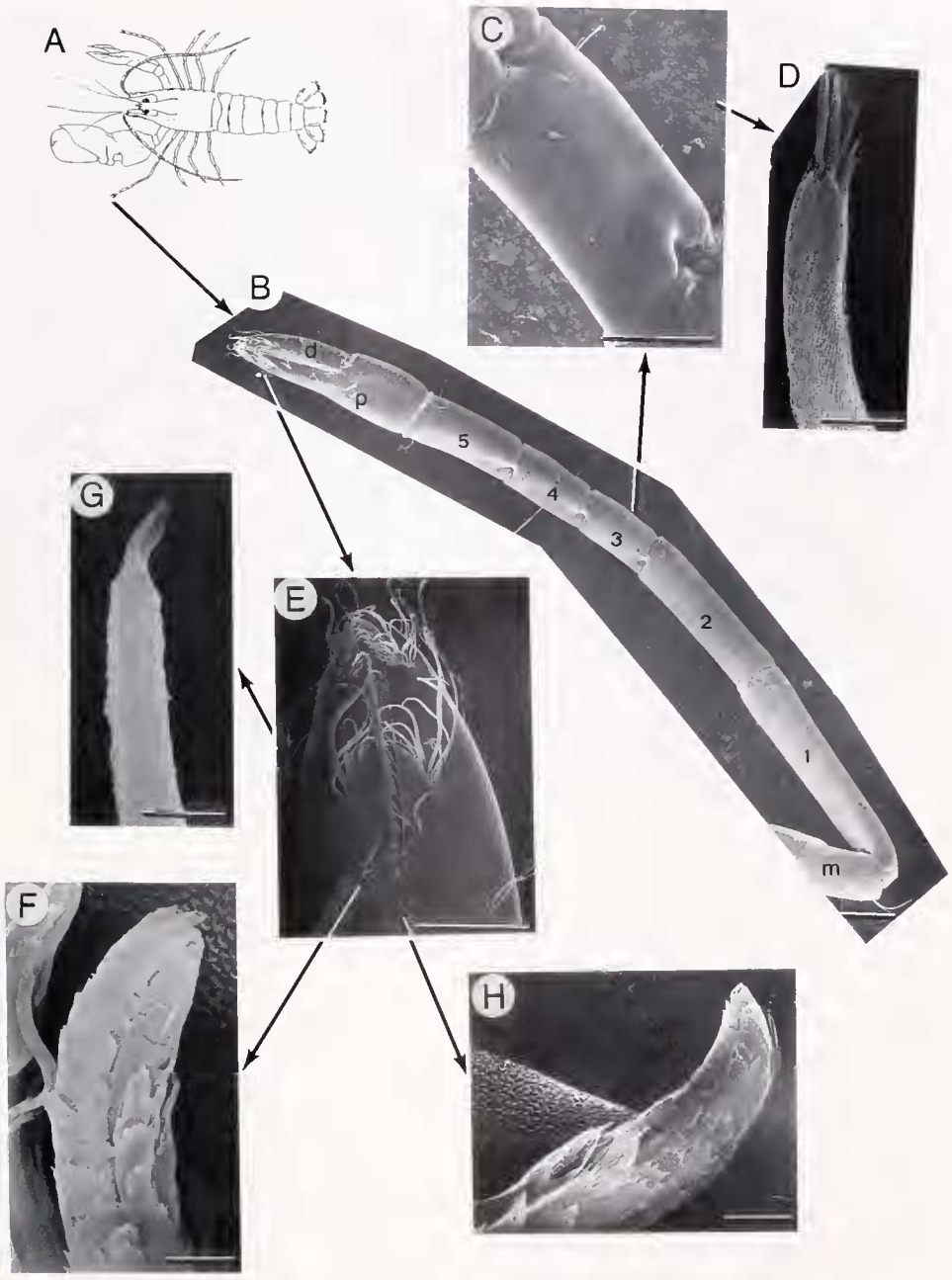

Figure 1. External morphology of the second thoracic limb of the snapping shrimp viewed with scanning electron microscopy. (A) Line drawing of the intact animal showing the five pairs of thoracic limbs, of which the first pair is the enlarged, bilaterally asymmetric chelipeds, and the second pair is the thin, elongated, chelated, and highly flexible limb. (B) The second thoracic limb with dactyl (d) and propus (p), 5-segmented (1 to 5) carpus and merus (m). Morphology and distribution of setae on this limb are shown in the accompanying figures. (C) A typical carpal segment with few sensilla which are of the setal type (D). (E) Distal end of the propus and dactyl with several clusters of long setae and a single row of short setae along the apposing edge of the dactyl. (F) Typical long setae showing serrulate nature and an apical pore. (G) Long setae with a sheath-like tip. (H) Typical short setae with serrulate form and apical pore. Scale bars: B, C, 500 μm; E, 250 μm; D, F, G, H, 2 μm.

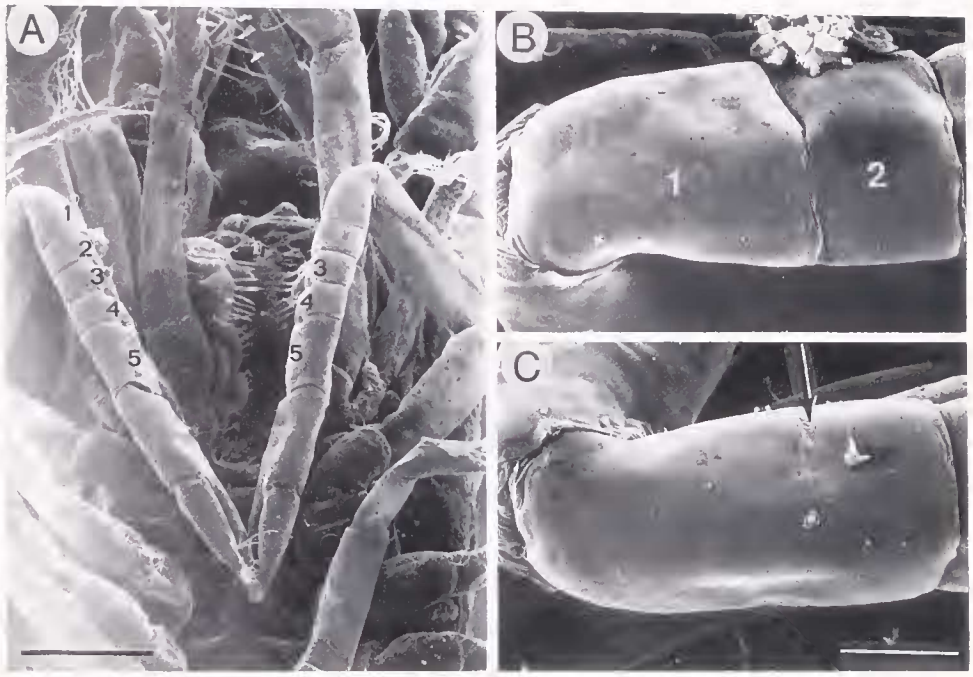

Figure 2. (A) External morphology of the paired second thoracic limbs in a juvenile shrimp. The carpus of the right limb is differentiated into five segments (1 to 5), while the left limb shows the most distal three segments (3 to 5) differentiated but not the most proximal two. (B) High power views of the most proximal segments (1, 2) of the right carpus while the corresponding region of the left carpus (C) is a single segment with a furrow (arrow) marking the beginning of segmentation into two. Scale bars: A, 200 μm; B, C, 50 μm.

closer muscle of the first thoracic limb (Phillips *et al.*, 1982). The nerve terminals were characterized by a population of clear synaptic vesicles that were spherical in most cases; occasionally, terminals with more irregularly shaped vesicles were encountered. The shape of these synaptic vesicles with aldehyde fixation—*i.e.*, spherical or irregular—effectively denotes excitatory or inhibitory nerve terminals (Atwood *et al.*, 1972).

The most distal carpal segment contains three muscles, a large stretcher muscle, a small bender muscle, and an even smaller rotator muscle (Fig. 3). The latter two muscles are closely juxtaposed and are situated in one compartment while the stretcher muscle lies by itself in the other compartment. The apodemes of these muscles attach to the next distal segment, although the muscles themselves do not traverse the full length of the segment but are restricted to the distal half. In fine structure these carpal muscles are similar to those in the propus, and they also display neuromuscular terminals indicative of innervation by both excitatory and inhibitory axons.

The musculature in each of the other segments of the carpus resembles that found in the most distal segment.

Neural elements. The second thoracic ganglion is consistently larger than the first (Fig. 4). In freshly dissected preparations from three adult shrimps, the surface area of the second ganglion was 25 to 30% larger than the first ganglion.

Differences in the input to the ganglia might account for the size differences between them. Hence we examined the nerves and connectives belonging to these ganglia.

A. Nerve. Each of the thoracic limbs is served by two separate nerves, the first and second nerve (Fig. 5A), which originate from the hemiganglion. Each nerve is mixed, composed of sensory and motor axons. The majority of axons within crustacean nerves are sensory, with their cell bodies located at the periphery (Bullock and Horridge, 1964). These sensory axons enter the ganglion and ramify in the neuropil, the integrative region of the ganglion. In crustaceans, relatively few (<60) motor axons innervate the limb musculature (Wiersma, 1961; Govind and Atwood, 1982).

The nerves of the snapping shrimp are unusual in that they have myelinated axons (Ritzmann, 1974), although peneid shrimp also have this feature (Heuser and Doggenweiler, 1966). Usually, however, crustacean nerves have only unmyelinated axons (Bullock and Horridge, 1964). Consequently in cross-sections, snapping shrimp nerves prominently display numerous, large Schwann cell nuclei characteristic of myelinated axons (Fig. 5A). At a higher magnification, myelinated axons are readily distinguishable from their unmyelinated counterparts, because the myelin forms a dense sheath around the axon (Fig. 5B). The unmyelinated axons are wrapped by glial sheaths to different degrees, depending on their size. The

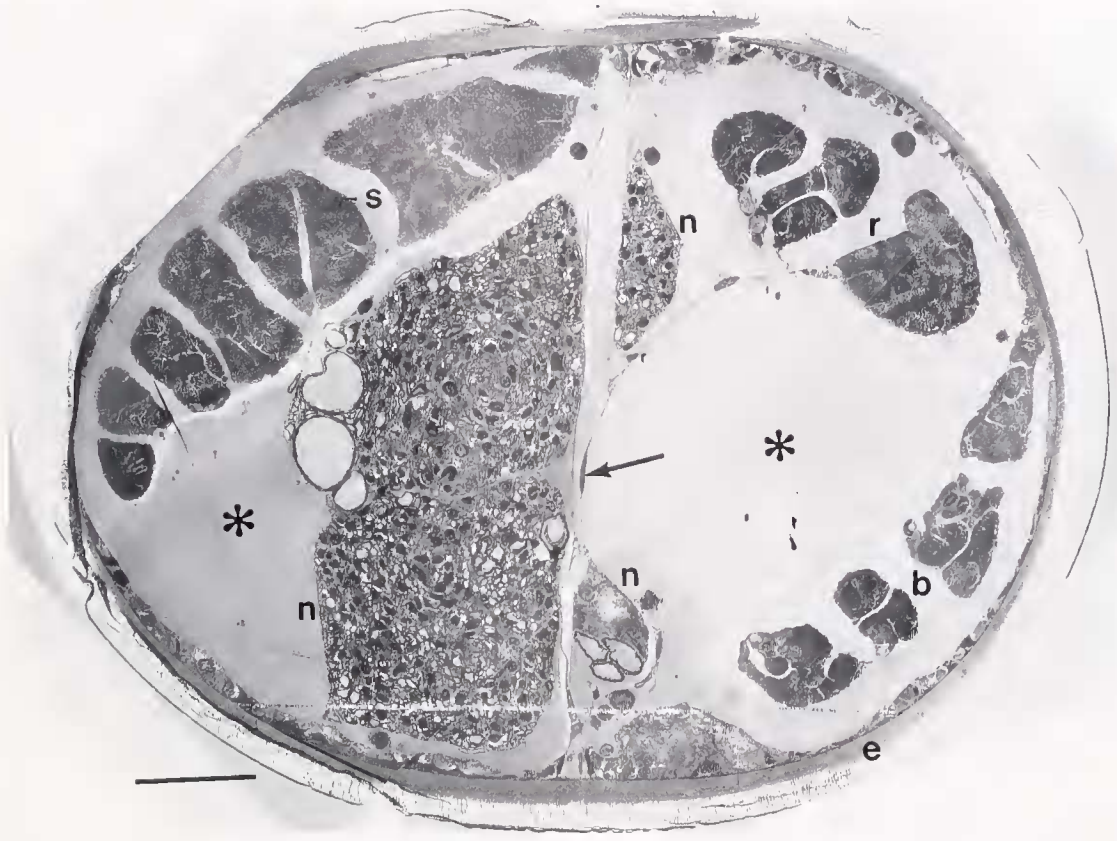

Figure 3. Cross-section through the first carpal segment with an exoskeleton (e) boundary and the interior separated into two compartments (asterisks) by a thin septum (arrow). One compartment has the stretcher (s) muscle and a large branch of the limb nerve (n) while the other compartment has the bender (b) and rotator (r) muscles and two smaller nerve (n) branches. Scale bar: 50 μm.

smaller axons are naked, whereas the larger axons have several layers of glial covering.

Because most of the axons in the nerves are sensory, we can estimate the sensory innervation from the limbs by counting the total number of axons close to the ganglion. Such counts were made in two animals, and the results were similar in both cases (Table I). The total numbers of axons on the right and left sides are almost equal. There is also an equal distribution between myelinated and unmyelinated axons on the right and left sides in each of the two animals.

To determine how the numbers of axons in the second limbs compare to those of the highly specialized and asymmetrical first thoracic limbs or chelipeds, counts were also made of the nerves to the pincer and snapper chelipeds (Table I). The present counts of numbers of axons to the snapper and pincer nerves confirms previous findings; the snapper side has more axons than the pincer side (Govind and Pearce, 1988).

Comparison between the first and second limbs reveals that the second limb has a much smaller number of axons than either the pincer or the snapper (Table I). In shrimp #1, for example, the second thoracic limb has 6000 axons, whereas the snapper on the first limb has over 13,000 axons and the pincer has 10,000 axons. In terms of total numbers of axons, the snapper has the largest number, followed by the pincer, and then the second thoracic limbs, which have the smallest number. The distribution of unmyelinated and myelinated axons is interesting; the first thoracic limbs (both the pincer and the snapper) have more (60–70%) unmyelinated than myelinated axons, whereas the second limbs have an equal number of myelinated and unmyelinated axons.

The above counts of axons taken close to the ganglion represented the total number to the limb, including those to the thorax at the base of the limb. Because we were interested largely in the multisegmented carpus, we counted the axons in the nerve running through the distal segments of a second thoracic limb. In all cases, counts were made from the most distal end of each segment. As anticipated, the axon number increased progressively, beginning at 1500 in the propus, to 1700 in the fourth carpal segment, to 2000 in the second carpal segment, to 2400 in the merus. The difference in number between the pro-

Figure 4. Photomicrograph of the thoracic nervous system in a freshly dissected shrimp, showing the paired hemiganglia to the first (1), second (2), third (3), and fourth (4) thoracic limbs, the nerves (arrows) from these hemiganglia and the opening (asterisk) for the dorsal artery in the connective between the third and fourth ganglia. Note the larger size of the second ganglia compared to the first or third. Scale bar: 250 μm.

pus and merus of 1000 represents the number of sensory axons originating in the multisegmented carpus.

B. Connectives. Apart from the nerves, the only other external source of neural input to the ganglia is via the connectives (Fig. 4). Therefore the number of axons that enter the ganglia via the connectives may be estimated by counting axon profiles in both the anterior and posterior connectives to the ganglion, as this would encompass both ascending and descending inputs. Consequently, counts were made in two animals, of the connectives anterior to the first, second, and third thoracic ganglia to estimate the anterior and posterior inputs to the first and second thoracic ganglia (Table II). The number of axons in the paired, left and right connectives at each of the three sampling stations were highly symmetrical. But the number in each of the three sampling stations was distinct, indicative of the relative degree of neural traffic to and from each of the first and second thoracic ganglia. Because the direction of the neural traffic—*i.e.,* whether it is ascending, descending or through-going—cannot be distinguished in these cross-sections of the connectives, the numbers from the anterior and posterior connectives were simply added as an estimate of the input into each of the first and second thoracic ganglia. The second thoracic ganglion had a higher number than the first, approximately 4000 *versus* 3000 axons, suggesting that the neural input is greater to the second thoracic ganglion than to the first.

Behavior

Both casual and formal observations of adult snapping shrimps in glass aquaria show the second thoracic limbs to be almost constantly active, primarily in probing the environment and, to a lesser degree, in grooming body parts. Probing is the rapid, jerky touching of the substrate or other objects by the chelae of the flexible limb. This is greatly facilitated by the multi-segmented carpus, which gives the limb enhanced flexibility, allowing it to probe deeply into the benthic crevices.

Probing occurs when the shrimp are crawling (backwards and forwards), burrowing (shovelling substrate with pereiopods, pleopod beating of substrate), or just standing still (including pleopod beating of the water).

Grooming involves picking at various parts of the body with the rapidly opening and closing chelae of the flexible limbs. Although virtually every part of the body is accessible, the most frequently groomed areas include the rostrum, gills, ventral thorax and abdomen (including pleopods), and the large chelae.

The flexible limb also retrieves and brings food to the mouth. Occasionally these limbs lifted and carried relatively heavy objects, such as large pebbles.

Time budget. As mentioned previously, one of the most striking aspects of the flexible limbs was their almost constant activity. This is reflected in the time budget analysis (Table III), which shows these limbs as being active 95% of the time and inactive only 5%. The majority (77%) of this activity was devoted to probing the substrate, be it sand or gravel. Moreover, immobilizing the carpal segments, by gluing them, had little effect on the probing activity. Probing occurred whether the shrimp was stationary, crawling, or burrowing, and the time devoted to this activity was approximately similar for each of the three states.

Grooming was the only other activity that occupied a significant amount of the time of the flexible limbs, although the time occupied (13%) was considerably smaller than that spent in probing. Grooming was confined principally to the head region.

Foraging experiments. The flexible limbs were actively engaged in foraging for food, the introduction of which caused very intense probing activity. When found, food was retrieved and brought to the mouthparts exclusively by the flexible limbs. Sometimes, the food was held by the pincer while bits were torn off by the flexible limb chelae, and the pincer was occasionally used to push the food into the sand. Rarely, jets of water produced by closure of the snapper were used to uncover food.

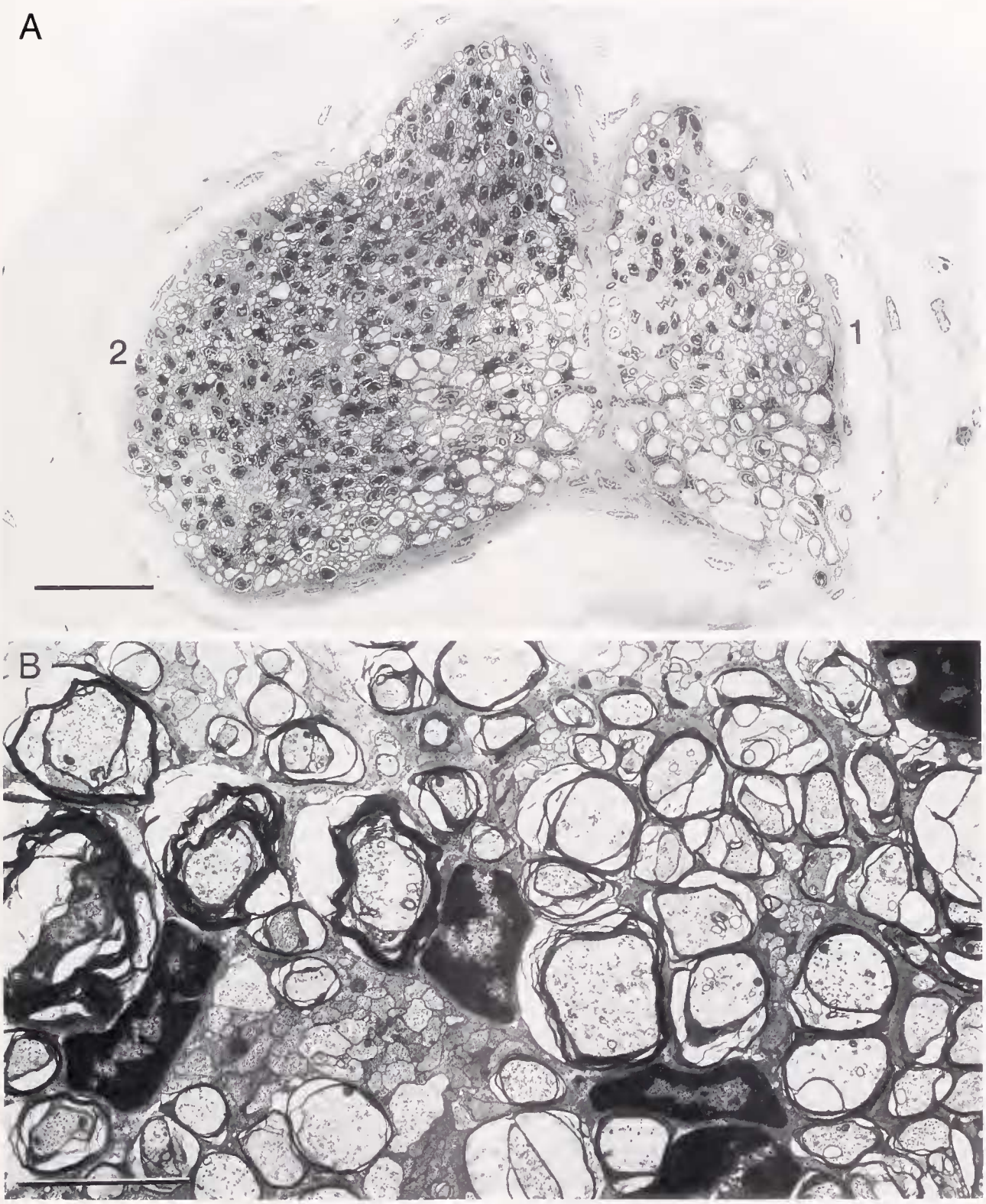

Figure 5. (A) Cross-section of the small first (1) and large second (2) nerves at the base of the hemiganglion to a second thoracic limb; each nerve has tightly packed axons and prominent nuclei of Schwann cells. (B) Representative area of the nerve cross-section showing clusters of small unmyelinated axons which are naked while myelinated axons have a densely stained sheath and scattered dense staining nuclei. Scale bars: A, 50 μm; B, 5 μm.

Table I

Number of axon profiles in the paired (right and left) nerves to the first and second thoracic limbs in two snapping shrimps

Thoracic limbs	Myelinated	Unmyelinated	Both
	Shrimp #1		
First thoracic limb			
right (pincer)	4071	6441	10512
left (snapper)	4286	8722	13008
Second thoracic limb			
right	3197	3072	6269
left	2885	3117	6002
	Shrimp #2		
First thoracic limb			
right (pincer)	3247	6214	9461
left (snapper)	4637	9232	13869
Second thoracic limb			
right	3451	3880	7331
left	3691	3791	7482

Table II

Number of axon profiles in the paired (left and right) connectives to the first and second thoracic ganglia in two snapping shrimps

Thoracic ganglia	Connectives		Total (right and left)	Total (anterior and posterior)
	left	right		
			Shrimp #1	
First thoracic	403	443	846	3293 to first ganglion
Second thoracic	1187	1260	2447	4169 to second ganglion
Third thoracic	876	846	1722	
			Shrimp #2	
First thoracic	486	454	940	3137 to first ganglion
Second thoracic	1087	1110	2197	4348 to second ganglion
Third thoracic	1120	1031	2151	

To evaluate the role of the second thoracic limbs in locating and retrieving food, these functions were compared in two groups of 10 shrimp each. The flexible limbs of the first group were intact; they were autotomized in the second group. In 21 separate trials, the intact shrimps were able to locate and retrieve the concealed food in all cases. The time taken to locate the food was 72 ± 18 s (SEM), and to retrieve it with the second thoracic limbs added only a few more seconds (80 ± 18 s SEM). Unexpectedly, the autotomized shrimps, in 17 out of 19 separate trials, successfully located the concealed food and the time taken was 115 ± 24 s (SEM) which was not significantly different (Students *t*-test) from the time for the intact group. The food location was done by frequent and direct contact of the substrate with the mouthpart region, *i.e.*, substrate pressing. However, once the food was located via such substrate pressing, the shrimps were unable to retrieve it because the second thoracic limbs were missing. Only in exceptional cases, and with the persistent use of other appendages, were the autotomized shrimps able to retrieve the food placed in the coral. Clearly, the second thoracic limbs were not required in locating concealed food, but were indispensable for retrieving it.

Conspecific interactions. To determine the role of the second thoracic limbs in conspecific interactions, observations were made of male/female and male/male pairs. The flexible limbs appeared to play little part in hetero- or homosexual interactions, and, in fact, during such encounters, the limbs were held reflexed posteriorly in a locked position, completely out of the way.

Discussion

The chitinized exoskeleton of crustaceans restricts flexibility within a limb to its segmental joints. Hence the unusual occurrence of a multisegmented carpus in the second thoracic limb of the snapping shrimp, *Alpheus heterochelis,* makes this limb particularly adept at exploratory behavior, food gathering, and grooming. Such behaviors occur constantly and are subserved by a ganglion which is larger than its neighbors. This size difference is related to the greater number of axon profiles in the longitudinal connectives to this ganglion than to the neighboring ganglia. The significance of these findings concerning the external and internal morphology of the second thoracic limbs and their behavior are discussed below.

Table III

Time budget for activities related to the second thoracic limbs in snapping shrimps

	% of time		
	Sand	Gravel	Overall
Probing:			
total	79.5	75.1	77.0
burrowing	16.8	32.3	24.6
crawling	34.1	20.5	27.3
stationary	28.6	22.2	25.2
Grooming:			
total	16.3	10.4	13.3
head	12.1	7.4	9.8
chelae	2.5	1.0	1.7
abdomen	1.7	2.0	1.9
Miscellaneous	3.0	6.7	4.8
Inactive	1.2	8.4	4.8
Number animals/observations	6/405	6/405	12/810

Morphology

The features of the second thoracic limb in the snapping shrimp, *Alpheus heterochelis*, that prompted this study were: its multisegmented carpus, its unusual flexibility, and its ganglion, which is larger than that of its neighbors. The first two are directly related, in that division of the carpus into five segments makes the limb highly flexible. The extent to which this specialization in the structure and function of the second thoracic limb regulates the size of its ganglion is somewhat more difficult to resolve. A simple possibility was that the input to the ganglion would regulate its size, and we tested this possibility by counting the number of axon in the limb nerve and the longitudinal connectives to this ganglion.

Numbers of axons in nerves. Cross-sections of nerves to the thoracic ganglia in several crustaceans reveal thousands of axon profiles of varying diameters, with the majority being small (<5 μ) (Sutherland and Nunnemacher, 1968; Govind and Pearce, 1985). Some of the large profiles are presumably motor axons, and these are comparatively few because crustacean limb muscles are innervated by only a few (1–5) motor axons (Govind and Atwood, 1982). For each of the distal segments of the limb—the merus, carpus, and propus—there are typically two antagonistic muscles, and each muscle is innervated by 2–5 axons; thus there are altogether about 30 axons. Another 30 motor axons would account for the more proximal muscles of the limb, making a total of 60 motor axons. This number is a very small percentage of the thousands of axons counted in the nerves.

The vast majority of axons in these nerves are presumably sensory. Consequently, counting axon profiles in these nerves provides an index of the sensory input to the ganglion. Furthermore, because the size of the ganglion will, in part, be governed by the sensory input, differences in the axonal counts between the first and second thoracic limbs may underlie the differences in size of the respective ganglia.

The correlation we find between axon numbers and size in the snapping shrimp hemiganglia is a negative one, however. The chelipeds, both of which have a substantially larger number of axons (9,000 and 13,000), are each associated with a smaller ganglion than that of the second thoracic limbs. Therefore, as a first approximation, the peripheral input to the ganglia cannot explain the size differences between the first and second ganglia.

Alternatively, the size differences between the ganglia might be attributed to the extra carpal segments in the second thoracic limb; there are five of these, compared to only one carpal segment in the chelipeds. Each of the five carpal segments in the second thoracic limb has at least three muscles, and they may receive their own motor axons. If this were the case, and assuming that each muscle receives a minimum of two axons, the extra segments would add an additional 24 motor axons. Because each motor axon extends a dendritic tree within the neuropil, it is likely that the addition of 24 motor axons would increase the size of the neuropil.

Numbers of axons in the connectives. Apart from peripheral nerves, the only other source of nerve input to the ganglia is via the connectives. The total input to each of the ganglia was estimated by simply adding the numbers of axons in the connectives anterior and posterior to each ganglion; ascending and descending input could not be distinguished. A positive correlation was seen between axon numbers and the size of the ganglia, as there were 4000 axons to the second thoracic ganglion, and 3000 axons to the first ganglion. This 25% difference is close to the 25–30% difference in the size of the hemiganglia.

In summary, the greater size of the second thoracic ganglion, relative to its first thoracic counterpart, is not simply due to differences in the number of sensory axons from the periphery to the ganglion. Consequently, it must be due to central factors. Finding a greater number of axons in the ventral connectives to the second thoracic ganglion, compared to the first, is consistent with this conclusion. The exact nature of these central features is unknown, but they may be related to the specialized nature of the second thoracic limb as a highly flexible mechanosensory "arm" capable of delicate movements. Consequently, we may anticipate additional motor axons, as in the extra carpal segments, a higher concentration of proprioceptors and, perhaps, a higher concentration of interneurons emanating from the ganglion. All of these features would contribute to increasing the size of the ganglion.

Behavior

Nolan and Salmon (1970) observed that *A. heterochelis* spent most of its active time grooming. In contrast, we found that the overwhelming majority of time was spent probing. Undoubtedly though, the flexible limbs are vitally important in both functions and are in almost constant motion, whether the shrimp is burrowing, crawling (walking), cleaning, feeding, or stationary. The ability to effectively "put them away" during agonistic encounters perhaps underscores their importance.

Food foraging. Animals that had autotomized their flexible limbs were at a great disadvantage in foraging for food. The ability to find concealed and hard-to-get-at food would obviously be beneficial in the coral reefs, oyster beds, and sponges frequently inhabited by *A. heterochelis* (Brooks and Herrick, 1892). Since alpheid shrimps spend much time actively browsing across the substrate, using their flexible limb chelae as probes and micromanipulators, this is likely an important means of foraging. In this

respect they are similar to prawns (Hindley and Alexander, 1978). However, alpheid shrimps are reported to stun prey such as other shrimps (McLaughlin, 1982) or to crack open clam shells by means of their snap. This would be another means of foraging, although the availability of such large prey would likely be insufficient to support a dense population of shrimps (Dahl, 1968).

Autotomized shrimp easily located concealed food, suggesting that the limbs are not important for olfaction. Indeed, the flexible limb has relatively few external sensilla, even taking into account their small size. Those seen were similar to sensilla observed on the chelipeds (Read and Govind, 1990). Functions of the flexible limb sensilla, probably related to foraging, may include contact chemoreception (*i.e.*, taste as opposed to olfaction), contact mechanoreception, and perhaps texture sensitivity.

Grooming. Grooming reduces the incidence of epizoic and sediment fouling, which could seriously affect the health and sensory and locomotory abilities of individuals and increase the mortality of brooding embryos (Bauer, 1975, 1979). *A. heterochelis*, in particular, is parasitized by a conspicuous epicaridean isopod. Seen in about 2% of captured specimens, this parasite lodges in the ventral surface of the abdomen (pers. obs.), undoubtedly impairing the reproduction, if not the health and the molt cycle of affected animals. Obviously, grooming would be important in discouraging this parasite. In addition, *A. heterochelis* hosts another parasite which was a sac-like organism filled with eggs and which lodges beneath the carapace in the thoracic region, an area which is subject to very frequent grooming. Molting, which occurs every 18–25 days, does not rid the shrimp of either the abdominal or thoracic parasites, thereby underscoring the importance of grooming.

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